Effects of Silkworm Pupae Protein and Active Peptides on Human Immunity

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Abstract. The escalating global population and the concomitant scarcity of protein resources have heightened the urgency to explore novel sources of protein. Insects, as a taxonomic class of organisms, are abundantly enriched with superior-quality protein and steered by this premise, they present a prodigious opportunity for human employment and scientific investigation in the realm of biologic resources. The larval stage of the domesticated silkworms, extensively cultivated for their silk, give rise to the universally consumed pupae in East Asia that augment the dietary provisions. This paper provides a comprehensive discussion of the immune-boosting, antibacterial, antitumor, and antioxidant properties of silkworm pupae proteins. Additionally, it explores the possible allergic reactions that may arise from silkworm consumption. Silkworm chrysalis protein, which contains a plethora of bioactive peptides such as AKPGVY and AAEYPA, offers a novel source of antioxidant peptides that have been shown to bolster immune function, enhance the body's capacity to fend off bacteria and viruses, reduce the risk of tumor development, and mitigate adverse effects such as free radical oxidative damage. However, it is important to note that some individuals may be susceptible to allergic reactions to silkworm pupae. Consequently, safety concerns related to the use of silkworm pupae proteins and active peptides should be taken into account.

Keywords: Insect protein; immune enhancement; human immunity; silkworm pupae protein.

1. Introduction

The burgeoning and proliferating global demand for sustenance is intricately and indissolubly connected and associated with the burgeoning and proliferating global populace, which is predicted and anticipated to succeed and attain nine billion by the year 2050. Insect proteins, by virtue of their innately and inherently occurring protein reservoirs, are considered and deemed to be a highly optimistic and auspicious resource for progression and growth. A multitude and plethora of food technologists and experts are thus ardently interested and fascinated in delving and adventuring into the potential and possibilities of edible insects [1]. Myriad and diverse research studies have evinced and demonstrated that these insects not only present and offer a rich and copious source of protein, vitamins, minerals, and fats, but also possess and exhibit higher and superior feed conversion efficiency in comparison and contrast to meat-based animal protein [2]. he consumption and ingestion of insects by human beings is not a novel or fresh phenomenon, having and exhibiting been a custom and practice since the early phases and stages of human evolution. In several and various nations, it is now an integral and innate constituent of customary and habitual dietary patterns and behaviors [3]. Notably, as of 2012, a surfeit and glut of nineteen hundred and more edible and palatable insect species have been documented, observed, and noticed across the globe and world [2].

The house silkworm (Bombyx mori) is universally nurtured as a highly treasured insect for its pupae and silk, both of which manifest significant pecuniary value [4]. Furthermore, it is noteworthy that silkworm pupae are a common delicacy in East Asian countries such as China, Japan, and Korea. In excess of two millennia, silkworm pupae have served as a medicinal and fortifying resource in traditional Chinese medicine [5]. Recent studies have identified various bioactive compounds with potential health benefits in silkworm pupae, including pupae proteins and hydrolyzed peptides. Silkworm pupae proteins are known to enhance immune function through immune boosting, antiviral, antitumor, and antioxidant activities. The ingestion of silkworm pupae, in accordance with scientific inquiry, has been deemed a risk-free practice, given its non-mutagenic and non-hepatotoxic consequences upon the human anatomy [6]. Subsequent, more rigorous investigation into the potential medicinal or nutritional worth of silkworm pupae, as a supplementary dietary matter, is necessary. Given that silkworm protein is rich in vital amino acids such as valine and methionine, it has been successfully incorporated in a diversity of formulations as a source of nutrition for human consumption. Moreover, the peptides and proteins found in hydrolyzed silkworm pupae display an assortment of immune function properties, including advantageous antioxidant effects [7]. These findings demonstrate the importance of silkworm pupae in enhancing human health and provide a promising avenue for future research.

However, the consumption of novel proteins, such as those found in silkworm pupae, has raised significant concerns regarding their potential to elicit food allergies. Adverse reactions such as dizziness, nausea, vomiting, and even severe allergic reactions in the form of anaphylaxis have been reported in individuals who have consumed silkworm pupae proteins [8]. Thus, the allergenicity of silkworm pupae has emerged as a topic of extensive investigation worldwide.

The present paper aims to investigate the potential roles of silkworm pupae proteins in immune enhancement, antibacterial, antitumor, and antioxidant activities. Moreover, this paper intends to evaluate and explore the allergenic properties of silkworm pupae.

2. Immunomodulatory Effects of Silkworm Pupae Protein

The immunomodulatory effects of the protein derived from silkworm pupae have been thoroughly examined in several animal models. A rat-based study revealed that administration of silkworm pupa proteins resulted in the enhancement of the immune response along with a boost in antioxidative activity in the skeletal muscle and liver tissues [9]. Additionally, a previously unidentified hexapeptide from silkworm pupae was discovered to induce splenocyte proliferation and possess immunomodulatory properties, suggesting its potential therapeutic value [10]. Another study using D-galactose-induced senescent mice demonstrated that silkworm pupae peptides alleviated symptoms of immune depression and improved oxidative damage in vivo [11].

Overall, these findings suggest that silkworm pupae proteins can enhance both specific and nonspecific immune functions, thereby improving the body's ability to combat foreign antigens.

3. Antitumor Effect of Silkworm Pupae Protein

The findings of Li et al. (2018) with respect to the isolation of protein from silkworm pupae and its subsequent hydrolysis by alkaline protease, manifested a marked significance by bringing into focus the enormous potential of silkworm pupae protein hydrolysate (SPPH) in terms of its ability to selectively impede the proliferation of human gastric cancer SGC-7901 cells through the intrinsic apoptotic pathway [12]. Similarly, the investigation carried out by Ye et al. (2023) noted the significance of silkworm carboxypeptidase inhibitors in arresting gastric cancer cell proliferation via stimulation of proto-oncogene c-Myc expression while simultaneously inhibiting the EGF/EGFR signaling pathway [13]. Complementary findings were posited by Mu et al. (2022), whose research demonstrated how hydrolysis of silkworm pupa protein could effectively curtail MGC-803 gastric cancer cell proliferation by inducing apoptosis in tumor cells [14].

The inquiry undertaken by Xu et al. (2020) encompassed an evaluation of the efficacy of BmAMP, viz. BmCecA and BmCecD, derived from silkworms, as a viable agent aimed at suppressing human esophageal cancer cells [15]. In point of fact, their research has evidenced the capacity of BmCecA and BmCecD to efficaciously hamper both the proliferation and migration of human esophageal cancer cells. It can be duly inferred from their study that the therapeutic use of BmCecA and BmCecD exhibits a remarkable potential in hindering the growth and advancement of human esophageal cancer cells. Moreover, BmCecA demonstrated the ability to incite apoptosis and was determined to be devoid of biochemical and physiological endangerment to organs outside of the diseased tissue.

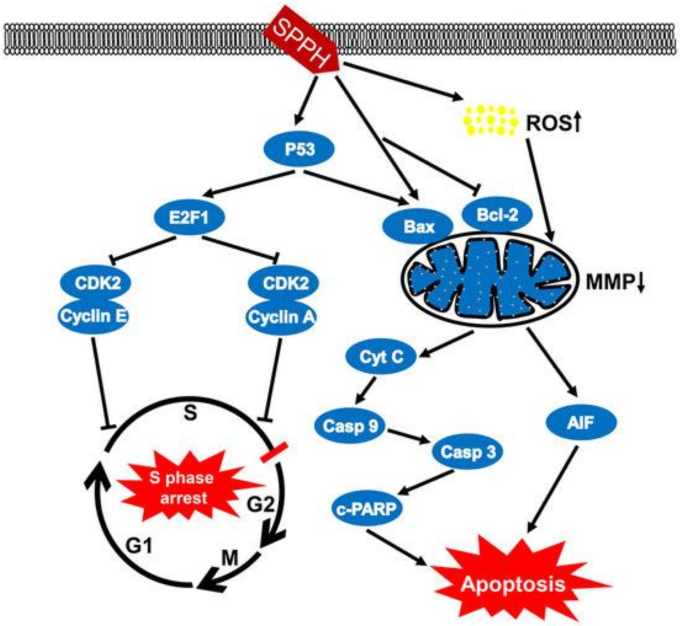

Fig 1. The model suggested for how SPPH induces cytotoxicity in SGC-7901 cells [12].

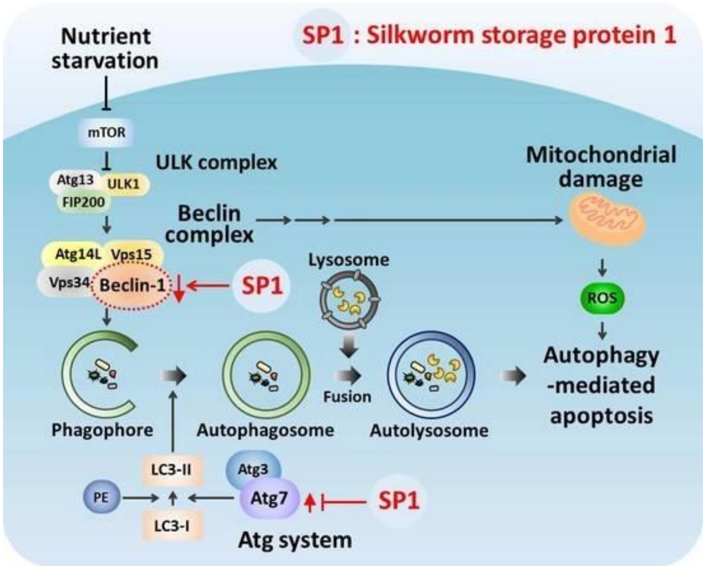

Fig 2. The anticipated biochemical pathway of storage protein 1 (SP1) in silkworms is associated with impeding autophagy-triggered programmed cell death caused by starvation [16].

4. Antibacterial Effect of Silkworm Pupae Protein

Insect-derived biomolecules have been proven to possess the potential to furnish bioactive peptides, inclusive of effective antimicrobial counterparts, by means of enzymatic protein hydrolysis - this is substantiated by several scientific inquiries. Among such a diverse population of protein-bundled entities that has been demonstrated to be capable of yielding new proteins at impressive levels are those which hail from sericultural chestnut-hued silkworm pupae, who induce production of these biomolecules upon induction by the bacterial strain Escherichia coli; this stimulates correspondent antimicrobial action against variegated bacterial species such as Bacillus subtilis, Staphylococcus aureus, Streptococcus serrata, and in conjunction with that, the aforementioned Escherichia coli [17]. The resounding findings of a scholarly evaluation reported by the renowned Singh et al. in the year 2014, are testament to the utilitarian value for domestic silkworms in a prokaryotic system for protein expression - this was vitalized in order to design serotonin 1 and 2 recombinant proteins; subsequently, experimentation detecting the antimicrobial potency of those proteins was conducted [18]. As a result, it was abundantly clear that serotonin 2 rendered potent effects on the pathogenic growth of gram-negative Escherichia coli and gram-positive Micrococcus luteus, as opposed to serotonin 1, which exclusively proffered inhibitory effects on the latter-described gram-positive luteus. In a recent investigation conducted by Zhu and colleagues in 2020, it was unearthed that the three serotonin varieties in silkworms, that is, serotonin, serotonin 1 and 2, and serotonin 3, demonstrated a parallel antibacterial mechanism. This process was accomplished via fastening the bacterial peptidoglycan utilizing the C-terminal structural domain, followed by the impeding of bacterial growth through the N-terminal structural domain [19]. These three distinct silkworm serotonins exhibited varied function in distinct domains, with silkworm serotonin 1 and 2 manifesting their antimicrobial properties in cocoon silk, and serotonin 3 in home eggs. In another independent inquiry, conducted by Kim and associates in 2019, the extracts of blood cells derived from the silkworm larvae, which were triggered by Lactobacillus cell wall extracts, were found to possess the ability to combat gram-negative bacteria, specifically Escherichia coli, by displaying anti-inflammatory effects over the LPS-induced THP-1 cells [20].

5. Antioxidant Activity of Silkworm Pupae Protein

The assessment of antioxidant potential in proteins originating from silkworm pupae and their corresponding hydrolysates has been widely examined. In a recent investigation, Sarkar et al. performed a separation procedure followed by proteolysis of non-mulberry silkworm pupa powder. Two species of silkworm pupae were chosen, Antheraea assama and Philosomia ricini. It was revealed that the resultant hydrolysates achieved the highest ACE inhibition and antioxidant attributes subsequent to treatment with alkaline protease (A. assama) and papain (P. ricini) for 60 and 240 minutes [21]. Cermeno et al. (2022) conducted a study to examine the efficacy of various protein hydrolysis preparations such as Alcalase (R), Prolyve (R), Flavorzyme (R), and Brewers Clarex (R) on the antioxidant activity of silkworm pupae hydrolysates. The experiment revealed that the use of Flavorzyme and Brewers Clarex (R) protein hydrolysis preparations showcased the most promising ACE inhibition and antioxidant properties [22]. The outcomes ascertained that the treatment of silkworm pupae proteins with Flavorzyme and Brewers Clarex protein hydrolysis preparations amplified ferric reducing antioxidant activity. Moreover, the Flavorzyme hydrolysates displayed a noteworthy decrease of 40% in reactive oxygen species in comparison to the untreated control HepG2 cells. The antioxidant activity of silkworm protein hydrolysates, prepared from alkaline protease, papain, and trypsin, claimed by Khammuang et al. (2022) fostered the safeguarding of DNA from hydroxyl radical damage [23]. Notably, the research work identified novel antioxidant peptides AKPGVY and AAEYPA from silkworm pupae protein hydrolysates. Moreover, these peptides exhibited the remarkable ability to restrain free radicals. In the context of clinical and life sciences, Choi et al. (2018) investigated the biopharmaceutical effects of steamed and lyophilized mature silkworm powder, and found that phytochemicals and silk proteins from silkworm powder were able to reduce reactive oxygen species produced by normal metabolism and environmental toxins in Drosophila [24]. This led to prolonged lifespan of Drosophila and inhibition of the onset of Parkinson's disease in Drosophila.

6. Allergenicity of Silkworm Pupae Protein

The safety of edible insects and potential allergic reactions associated with their consumption are currently major concerns among food safety scientists and consumers, especially in countries where eating insects is a cultural and culinary tradition. The safety of edible insects is a critical factor in ensuring their quality and building confidence in their consumption. However, the presence of numerous allergens in silkworms and their metabolites, including excreta, scales, and silk, raises concerns about their safety.

Silkworm-induced allergies may manifest as food allergies, respiratory allergies, and contact allergies [25]. In East Asian countries, consumption of silkworm pupae is a common cause of allergic reactions [26]. A number of cases of allergy related to the consumption of silkworm pupals have been reported in China [27]. And in Korea, Kim et al. (2003) reported allergies to silkworms in 9.4% of patients [28]. During the breeding of silkworms, airborne silkworm moth scales may be inhaled and cause asthma [29]. Silkworm quilts are also widely used, and contact allergy may occur during their processing and use in individuals allergic to silkworms or silk products [24]. Therefore, allergens may be present at different developmental stages of the life cycle of domestic silkworms.

Forty-five potential allergens harbored in domestic silkworms have been determined by scientists. From these, a total of twenty-six silkworm pupae proteins have been identified to act as allergens. However, only a selective five among the allergenic proteins derived from silkworm moths, which specifically include Bom m1 (arginine kinase), Bomb m3 (tropomyosin), Bomb m4 (30 kDa Hemolymph Lipoprotein), Bomb m5 (30 kDa Lipoprotein), and Bomb m6 (Hemolymph lipoprotein 3), have been certified by the WHO/IUIS Sub-Committee on Allergen Nomenclature [30]. It is of remarkable significance to point out that arginine kinase, an enzyme responsible for the transfer of high-energy phosphate groups within cells, has been apprehended as an allergen solely in the larvae of silkworms, but not in their pupal form. In accordance with the assertions propounded by Wang and colleagues, the genetic expression rate of arginine kinase in the pupal stage of the silkworms manifested a conspicuously low value. Nevertheless, the same was observed to escalate considerably during the maturation of the larvae [31]. Thus, further scrutiny is warranted to determine the allergenic nature of arginine kinase in domestic silkworms' pupae.

Furthermore, 11 new potential allergens were identified in the metabolites of domestic silkworms, and some potential allergens of domestic silkworms have high homology to known allergens. Cross-reactivity of silkworms with these allergens may be an important cause of allergic diseases; however, further studies are necessary to confirm this hypothesis.

7. Conclusion

Silkworms are recognized as one of the most economically valuable insects because of their production of silk threads from cocoons. The textile industry generates silkworm pupae as a byproduct, which have potential applications as both food and medicine. Extensive research has been conducted on silkworm pupae, and several studies have highlighted their nutritional and functional benefits for human consumption. Active substances in silkworm pupae, particularly pupae proteins and hydrolyzed peptides, have various biological activities, such as immune-boosting, antiviral, antitumor, and antioxidant effects, which can enhance human health and well-being.

However, the utilization of silkworm pups as a sustainable food source requires a comprehensive risk assessment to identify potential allergy risks. Although the available data on this topic is limited, allergenic proteins and peptides, namely arginine kinase and tropomyosin, have been identified in silkworms. Exposure to silkworms can lead to allergic reactions via ingestion, inhalation, or contact routes. Hence, further research is imperative to evaluate the safety of silkworm and its byproducts consumption, and to formulate measures aimed at mitigating the risk of allergic reactions.

To evaluate the safety of silkworm pupae-derived chrysin and hydrolyzed peptides as promising food ingredients, a comprehensive assessment is necessary, despite numerous studies demonstrating their safety. Although no significant toxic side effects have been reported, safety concerns should be addressed to ensure their safe application. It is anticipated that future studies will explore the mechanisms of action of pupal proteins and hydrolyzed peptides to maximize their functionality while minimizing adverse effects.

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